British Heart Journal, 1977, **39**, 29–34

Excretion of myoglobin in urine after acute myocardial infarction

T. G. DONALD, M. J. CLOONAN, CYNTHIA NEALE, AND D. E. L. WILCKEN

From the Divisions of Cardiovascular Medicine and Microbiology, Prince Henry Hospital, Little Bay (Sydney), New South Wales, 2036, Australia

We studied myoglobin excretion in 33 patients admitted to the coronary care unit with a provisional diagnosis of acute myocardial infarction. Sixteen proved to have definite and uncomplicated acute myocardial infarction and 17 possible infarction, using WHO criteria. For 5 days after admission, aliquots of every urine specimen voided by each patient were analysed for myoglobin using an immunochemical method able to detect a minimum urinary myoglobin concentration of 0·02 mg/ml. Myoglobinuria was detected in 14 of the 16 patients with definite infarction but was not found in any of the 17 patients with possible infarction. There were 3 patterns of myoglobin excretion. In 8 of the 14 patients it was excreted in one episode starting 10 to 40 hours after the onset of chest pain and lasting for 5 to 45 hours. In 3 of the remaining patients it was excreted over a much longer period (mean 83 hours) and in the final 3 patients myoglobinuria occurred in 2 or 3 intermittent episodes with periods of between 10 and 20 hours during which it was not detected. Total myoglobin excretion, which varied between 2 and 100 mg (mean 51 mg), did not correlate with peak serum enzyme levels. We concluded that in the appropriate clinical setting, the finding of myoglobinuria provides additional evidence for a diagnosis of acute myocardial infarction. The variable myoglobin excretion pattern suggests that in seemingly uncomplicated myocardial infarction there is considerable variation between patients in the pattern of evolution of the infarction process. This may be relevant to the assessment of measures directed towards limiting infarct size.

Confirmation of a clinical diagnosis of acute myocardial infarction is sometimes elusive, as non-specific changes in the electrocardiogram are not infrequent and difficult to interpret in the absence of clear-cut increases in serum enzymes (Friedberg, 1966; Sobel and Shell, 1972; Nevins *et al.*, 1973). Patients with recent chest pain and an old infarct pattern or left bundle-branch block on the electrocardiogram are examples of this. It has been shown recently that after acute myocardial infarction, myoglobin is released from damaged heart muscle into the blood stream and is then cleared rapidly from the circulation and excreted in the urine (Kagen, 1973). Several studies have indicated that the detection of myoglobin in the urine may be a useful additional test in the diagnosis of acute myocardial necrosis in man (Strausser, Rothfeld, and Bucsi, 1966; Adams and Elliott, 1970; Levine *et al.*, 1971; Saranchak and Bernstein, 1974; Kessler *et al.*, 1975). If the total amount is related to the amount of muscle necrosis, then measurements of myoglobinuria could be relevant to an understanding of the evolution of the infarction process.

Received for publication 8 July 1976

We have recently developed a simple and sensitive method for the immunochemical detection of myoglobin in human urine (Cloonan *et al.*, 1976). We undertook the present study to determine the time course of myoglobinuria after acute myocardial infarction and to quantify myoglobin excretion. This paper reports our findings.

Subjects and methods

We studied 33 patients admitted to the coronary care unit with a provisional diagnosis of acute myocardial infarction. The average time from the onset of chest pain to admission was 3 hours. A standard 12-lead electrocardiogram was performed daily on each patient for 3 consecutive days and analysed for definite myocardial infarction, possible infarction, or no infarction, according to WHO criteria (1971). Venous blood was taken daily for the first 3 to 5 days for estimation of creatine kinase (CK—normal range 5 to 50 U/l) and hydroxybutyrate dehydrogenase (HBD—normal

range < 250 U/l). Both were estimated kinetically at 37°C and 340 nm wave length. Raised levels considered consistent with myocardial infarction were greater than twice the upper limit of normal for CK, and greater than 250 U/l for HBD, the levels of each increasing and declining appropriately with time after the onset of chest pain (Friedberg, 1966). Serum creatinine was measured daily (normal range 0·01 to 0·11 mmol/l). All patients were uncomplicated in that none developed ventricular fibrillation or ventricular tachycardia or required treatment for hypotension.

On the basis of the history, the electrocardiogram, and the serum enzyme levels, the 33 patients had either definite acute myocardial infarction or possible acute myocardial infarction according to WHO criteria (1971). For a period of 5 days after admission, an aliquot of every urine specimen voided by each patient was immediately frozen and stored at -20°C for later myoglobin estimation. The volume of each voiding was recorded.

For the myoglobin estimation, urine specimens were thawed and an aliquot centrifuged for 10 minutes at 1000 g. The supernatant was tested for myoglobin on the same day. The method used has been fully described elsewhere and will be reviewed only briefly here (Cloonan *et al.*, 1976). The only difference was that in the present study we used myoglobin antiserum from a commercial source (Hoechst, Behringwerke AG, Marburg-Lahn) which gave results similar to those reported previously (Cloonan *et al.*, 1976). Fowl red blood cells, to which purified human cardiac myoglobin had been attached, were agglutinated by specific myoglobin antibody. The presence of myoglobin in urine was shown by the inhibition of agglutination of the sensitised red blood cells as a result of myoglobin reacting with the specific antibody before the addition of cells. The minimum urinary myoglobin concentration measured by this haem-agglutination method was 0·02 mg/ml.

Results

Of the 33 patients with chest pain of ischaemic type, 16 had acute myocardial infarction and 17 possible infarction based on WHO criteria (1971). Myoglobin was detected in the urine of 14 of the 16 patients with acute myocardial infarction. It was not found in the urine of any of the 17 patients with possible infarction.

The Table summarises for each of the 16 patients with infarction, the site of infarction, peak serum enzyme levels, and the total amount of myoglobin excreted as well as the time of onset and of cessation of myoglobin excretion. In 9 of the 14 patients with myoglobinuria, myoglobin was detectable within 24 hours of the onset of chest pain. In 4 it was first detected during day 2, and in 1 for the first time during day 3. The total amount of myoglobin excreted in the 16 patients with infarction was poorly correlated with peak serum enzyme levels, as can be seen from the Table. However, the two patients without myoglobinuria had lower peak enzyme levels than did the 14 patients in whom it could be detected. Mean peak CK and HBD levels $\pm$SE for the latter were 832 ± 164 and 1048 ± 137 U/l, respectively. The corresponding values in the negative patients were 380 and 286, and 495 and 385 U/l (Table).

Table *Clinical and biochemical features of patients with acute myocardial infarction*

Case No.	*Site of infarct on electrocardiogram*	*Peak CK* (U/l)*	*Peak HBD* (U/l)*	*Total amount of myoglobin excreted (mg)*	*Onset of myoglobinuria (hours after onset of chest pain)*	*Cessation of myoglobin excretion*
1	Inferior	420 (24)	765 (24)	2	23	29
2	Anterior	255 (6)	270 (55)	96	35	108
3	Anteroseptal	675 (36)	2175 (36)	42	15	20
4	Inferior	1450 (31)	1700 (55)	30	4	70
5	Anterior	495 (10)	610 (30)	15	43	65
6	Anterolateral	495 (50)	1340 (50)	24	10	84
7	Anteroseptal	900 (15)	1040 (15)	34	30	60
8	Inferior	2000 (26)	1575 (50)	2	16	28
9	Anterior	365 (17)	600 (41)	≥99	10	≥132
10	Inferior	995 (24)	830 (47)	64	50	112
11	Anterior	2160 (40)	1250 (40)	100	10	45
12	Inferior	435 (16)	1050 (60)	48	14	59
13	Anterior	565 (48)	700 (47)	≥90	8	≥55
14	Anterior	440 (50)	765 (73)	64	28	49
15	Anterior	380 (36)	495 (36)	—	—	—
16	Anterior	286 (20)	385 (20)	—	—	—

*Figures in brackets represent time of enzyme estimations in hours after the onset of chest pain. In Case 13 urine collections were stopped prematurely; myoglobin was detected in the last urine sample available for testing in this patient and in Case 9 at 55 and 132 hours after the onset of pain, respectively.

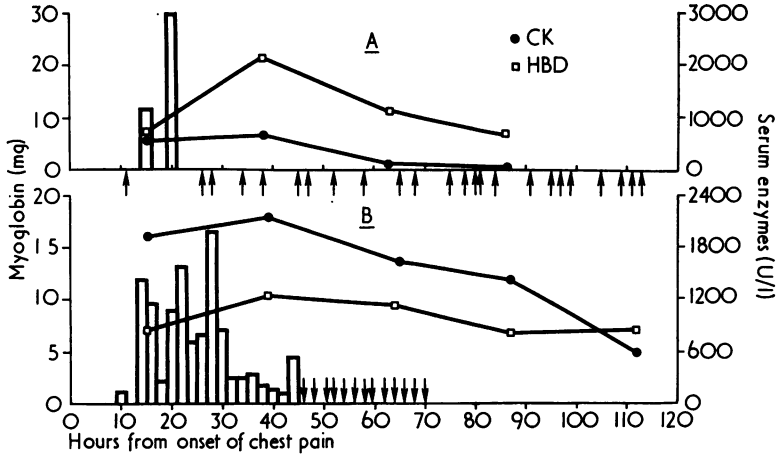

Fig. 1A and B *Urinary myoglobin excretion and serum enzyme levels in 2 patients with acute myocardial infarction in whom myoglobin was excreted in one episode. Vertical bars represent the total amount of myoglobin excreted in each urine specimen. Arrows indicate urine specimens in which myoglobin was not detected.*

There were three patterns of myoglobin excretion in the 14 patients. In 8 patients myoglobin was excreted in the urine in one episode starting 10 to 40 hours after the onset of chest pain and lasting for 5 to 45 hours (mean 23 hours). Fig. 1 illustrates the excretion pattern in 2 of the 8 patients showing the time and amount of myoglobin excreted in each voiding and levels of serum enzymes; the arrows indicate the time of voidings in which myoglobin was not detected.

In 3 of the 14 patients myoglobin was excreted over a much longer period. In these, it began 10 to 50 hours after the onset of chest pain and continued for between 60 and 120 hours (mean 83 hours). This prolonged excretion pattern is illustrated for 1 of the 3 patients (Case 2) in Fig. 2. In the final 3 patients, the pattern was again different. Myoglobin was excreted in 2 or 3 intermittent episodes. Excretion began 5 to 10 hours after the onset of symptoms and extended for between 70 and 80 hours, with periods of between 10 and 20 hours, during which no myoglobinuria could be detected. The excretion pattern found in one such patient (Case 4) is illustrated in Fig. 3.

Discussion

The finding of myoglobin in the urine correlated

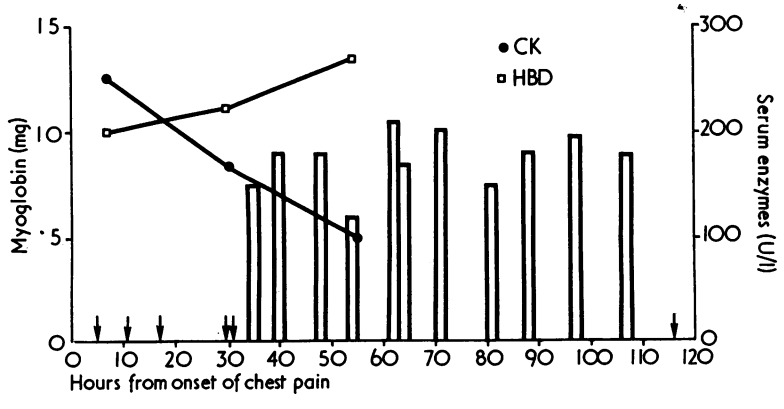

Fig. 2 *Urinary myoglobin excretion and serum enzyme levels in a patient with acute myocardial infarction illustrating a pattern of prolonged myoglobin excretion with only modest increases in serum enzymes. The vertical bars and arrows are as in Fig. 1.*

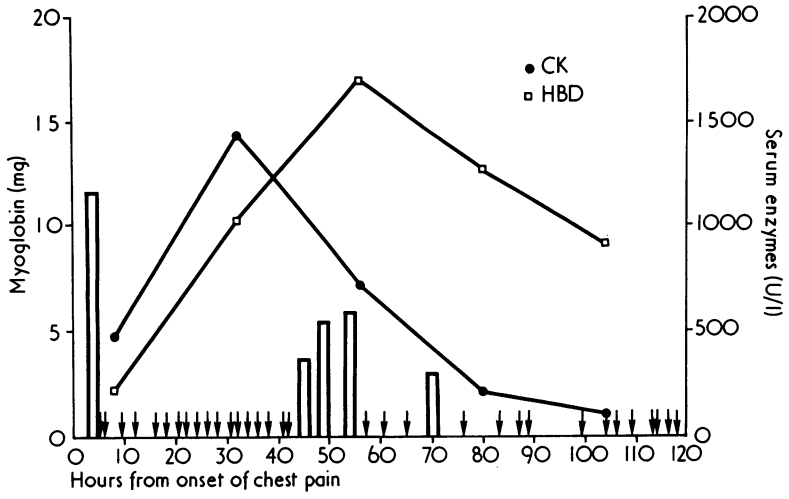

Fig. 3 *Urinary myoglobin excretion and serum enzyme levels in a patient with acute myocardial infarction illustrating an intermittent pattern of myoglobin excretion. The vertical bars and arrows are as in Fig. 1 and 2.*

well with the presence of definite acute infarction since it was detected in 14 of 16 such patients and in none of the 17 in whom the diagnosis was in doubt (possible infarction). Tests for myoglobin do not distinguish between that of skeletal or cardiac muscle origin. In our earlier study (Cloonan *et al.*, 1976) involving 147 control patients, and 15 patients given intramuscular injections similar to those received by the patients of the present series, no patient had myoglobinuria after an injection; and only 4 of the 147 controls had detectable myoglobinuria, and each had a condition likely to produce dysfunction of skeletel muscle cells. Thus, in the present study, we concluded that the myoglobin came from damaged heart muscle; and that in the appropriate clinical setting, the finding of myoglobinuria provides confirmatory evidence for a diagnosis of acute myocardial infarction. Using a method capable of detecting myoglobin in concentrations of 0·02 mg or more per ml of urine, myoglobinuria occurs regularly after acute myocardial infarction, being present in 87 per cent of patients in the present series.

A feature of the studies of myoglobinuria after myocardial infarction reported so far has been the variability of results; in some studies significant numbers of control patients had myoglobinuria (Strausser *et al.*, 1966; Adams and Elliott, 1970). In several studies, single daily samples only were tested (Levine *et al.*, 1971; Saranchak and Bernstein, 1974). Kessler and his associates (1975) examined samples of all urine passed in the first 24 hours, with analysis of single random samples over the next 3 days. However, to assess the significance of myoglobinuria after myocardial infarction it seems mandatory to analyse all urine samples voided for a considerable time after the onset of symptoms using a method of known sensitivity. From our preliminary studies, a period of 5 days seemed appropriate (Cloonan *et al.*, 1976).

The present study also shows that the excretion of myoglobin in the urine is extremely variable after acute myocardial infarction. While 8 of the 14 patients excreted it in one episode starting 10 to 40 hours after the onset of symptoms and lasting for a mean of 23 hours, in others excretion was intermittent or very prolonged—up to 132 hours after the onset of chest pain; and 11 of the patients had at least one myoglobin negative specimen 1 to 17 hours before the onset of myoglobinuria. From these findings it is clear that testing of single urine specimens soon after infarction, or even single daily urine specimens, will lead to false negative results. We could find no clinical, electrocardiographic, or serum enzyme evidence of delayed or recurrent infarction in individual patients to explain the variability we observed. It is to be emphasised that the present study was concerned only with patients with uncomplicated myocardial infarction or suspected myocardial infarction. In none were there serious arrhythmias, apart from ventricular ectopic beats; nor were there episodes of hypotension or of low cardiac output states detectable clinically. In no patient was there evidence of renal impairment as judged by an abnormal serum creatinine level. It certainly seems unlikely that the

variability of the myoglobinuria was related to undetected haemodynamic disturbances affecting the renal handling of myoglobin.

There is in the data a suggested relation between extent of necrosis and myoglobinuria in that no myoglobinuria was found in the patients with suspected infarction, some of whom may well have had small infarcts; and the 2 patients with definite infarction who were negative for myoglobinuria had lower peak serum enzyme levels than did the positive patients. It might have been expected that total myoglobin excretion would correlate well with the size of the infarct and that the extent of the enzyme rises would also be a reflection of this. But there was considerable variability between patients in the total amount of myoglobin excreted. Including the 2 patients in whom an end-point was not obtained, mean excretion was 51 $\pm$9 mg ($\pm$SE) with a range of 2 to 100 mg (Table). This variability was not well correlated with the peak serum enzyme levels—see the Table; the discordance is well illustrated in the results obtained in Case 2, shown in Fig. 2.

There are several possible explanations for these findings. Attempts to measure infarct size have involved estimating total CK release (Sobel *et al.*, 1972; Norris *et al.*, 1975). This was not possible in the present study. Serum enzymes were estimated only once daily and the measured peak values may well have been quite different from the true peak values and thus poor indications of extent of infarction. A more likely explanation in accordance with the present findings of variable myoglobin excretion pattern is that there is considerable variation between patients in the pattern of evolution of the infarction process. There is evidence for this proposition in several recent studies showing that in at least 30 per cent of patients with acute myocardial infarction the typical pattern of enzyme release with a steep rise to a peak at 18 to 48 hours (depending on the enzyme) does not occur (Reid *et al.*, 1974; Mathey *et al.*, 1975; Thompson, 1976).

Myoglobin (molecular weight=17 000) is a much smaller molecule than either creatine kinase or hydroxybutyrate dehydrogenase and should move more readily through damaged cell membranes. Recently Kagen and his associates (1975), using a complement fixation technique, were able to demonstrate myoglobin in the serum of 11 of 21 patients after acute myocardial infarction. Even more recently, and after completion of the present study, Stone and his associates (1975) reported the development of a very sensitive radioimmunoassay. Using this, they detected myoglobin in sera from normal adults and established that levels were greatly increased in 18 of 20 patients with acute myocardial infarction. In the future, measurements of levels in blood and urine may provide much more sensitive indices of myocardial damage than those currently available. They may well make it possible to explore the evolution of the infarction process with greater precision than at present. There is now good evidence, supported by the present study, that patients with seemingly uncomplicated myocardial infarction differ considerably in the rate with which they develop and complete the changes of cell dissolution and necrosis (Reid *et al.*, 1974; Mathey *et al.*, 1975; Thompson, 1976). An understanding of the mechanisms responsible should contribute to the assessment and rational use of measures directed towards limiting infarct size.

We thank Dr. David Brender for reviewing the manuscript.

References

Adams, E. C., and Elliott, T. A. (1970). Urinary myoglobin in myocardial infarction. *Journal of the American Medical Association,* **211,** 1013.

Cloonan, M. J., Donald, T. G., Neale, C., and Wilcken, D. E. L. (1976). The detection of myoglobin in urine and its application to the diagnosis of myocardial infarction. *Pathology,* **8,** 313.

Friedberg, C. K. (1966). Acute coronary occlusion and myocardial infarction. In *Diseases of the Heart,* 3rd ed., p. 866. W. B. Saunders, Philadelphia.

Kagen, L. J. (1973). *Myoglobin: Biochemical, Physiological and Clinical Aspects,* p. 79. Columbia University Press, New York.

Kagen, L., Scheidt, S., Roberts, L., Porter, A., and Paul, H. (1975). Myoglobinemia following acute myocardial infarction. *American Journal of Medicine,* **58,** 177.

Kessler, H. A., Liebson, P. R., Mattenheimer, H., and Adams, E. C. (1975). Acute myocardial infarction diagnosed by myoglobinuria. *Archives of Internal Medicine,* **135,** 1181.

Levine, R. S., Alterman, M., Gubner, R. S., and Adams, E. C. (1971). Myoglobinuria in myocardial infarction. *American Journal of the Medical Sciences,* **262,** 179.

Mathey, D., Bleifeld, W., Buss, H., and Hanrath, P. (1975). Creatine kinase release in acute myocardial infarction: correlation with clinical, electrocardiographic, and pathological findings. *British Heart Journal,* **37,** 1161.

Nevins, M. A., Saran, M., Bright, M., and Lyon, L. J. (1973). Pitfalls in interpreting serum creatine phosphokinase activity. *Journal of the American Medical Association,* **224,** 1382.

Norris, R. M., Whitlock, R. M. L., Barratt-Boyes, C., and Small, C. W. (1975). Clinical measurement of myocardial infarct size. *Circulation,* **51,** 614.

Reid, P. R., Taylor, D. R., Kelly, D. T., Weisfeldt, M. L., Humphries, J. O., Ross, R. S., and Pitt, B. (1974). Myocardial infarct extension detected by precordial S-T segment mapping. *New England Journal of Medicine,* **290,** 123.

Saranchak, H. J., and Bernstein, S. H. (1974). A new diagnostic test for acute myocardial infarction. *Journal of the American Medical Association,* **228,** 1251.

Sobel, B. E., Bresnahan, G. F., Shell, W. E., and Yoder, R. D. (1972). Estimation of infarct size in man and its relation to prognosis. *Circulation,* **46,** 640.

Sobel, B. E., and Shell, W. E. (1972). Serum enzyme determinations in the diagnosis and assessment of myocardial infarction. *Circulation*, **45**, 471.

Stone, M. J., Willerson, J. T., Gomez-Sanchez, C. E., and Waterman, M. R. (1975). Radioimmunoassay of myoglobin in human serum: results in patients with acute myocardial infarction. *Journal of Clinical Investigation*, **56**, 1334.

Strausser, H. R., Rothfeld, E. L., and Bucsi, R. A. (1966). Isolation and preservation of human myoglobin for use in immunologic detection of myoglobinuria. *Proceedings of the Society for Experimental Biology and Medicine*, **122**, 621.

Thompson, P. L. (1976). Measurement and limitation of infarct size: clinical application. *Australian and New Zealand Journal of Medicine*, in the press.

W.H.O. Regional Office for Europe (1971). *Report of 5th Working Group on Ischaemic Heart Disease Registers*, p. 28. Copenhagen.

Requests for reprints to Dr. D. E. L. Wilcken, Department of Medicine, Prince Henry Hospital, Little Bay, N.S.W., 2036, Australia.